

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

CRFE  
SEARCH REQUEST FORM

ACC DB# 99422

Scientific and Technical Information Center

Requester's Full Name: Patrick Lewis Examiner #: 79002 Date: 7-22-03  
 An Unit: 1623 Phone Number: 301-54043 Serial Number: 09/485,071  
 Mail Box and Bldg/Room Location: CM1/8012 Results Format Preferred (circle): PAPER DISK E-MAIL  
CM1/8-B-A

If more than one search is submitted, please prioritize searches in order of need.

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc. if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Modulators of DNA Cytosine-S-methyltransferase and  
Methyle for use Therap

Inventors (please provide full names): Herbert D. Reich ; James Flynn

Earliest Priority Filing Date: 8-29-1997

\*For Sequence Searches Only\* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

\* Bib Data Sheet attached

A synthetic oligonucleotide of at least 26 nucleotides in length and comprising a SmCpG dinucleotide, wherein the SmC is a C-S methylcytosine, and which comprises a nucleotide sequence SEQ ID NO: 10

CTGGATCCTTGGCCCCGCCCC  
 TTGAATTCCC

PLEASE Zush  
 Putrell

seq10 NA 30

STAFF USE ONLY

| Type of Search                         |   | Vendors and cost where applicable |
|--|---|-----------------------------------|
| Searcher: <u>D. Schreiber</u>          | NA Sequence (#) _____                             | STN: <u>108,50</u>                |
| Searcher Phone #: <u>308-4292</u>      | AA Sequence (#) _____                             | Dialog: _____                     |
| Searcher Location: <u>CM1 6A03</u>     | Structure (#) _____                               | Questel Orbit: _____              |
| Date Searcher Provided: _____          | Bibliographic <input checked="" type="checkbox"/> | Dr. Link: _____                   |
| Date Completed: <u>7/24</u>            | Citation: _____                                   | John's News: _____                |
| Searcher Prep & Review Time: <u>15</u> | Fulltext: _____                                   | Sequence Systems: _____           |
| Client Prep Time: _____                | Patent Family: _____                              | WWW Internet: _____               |
| Fee Paid: <u>46</u>                    | Other: _____                                      | Other Specialty: _____            |

Lewis 09/485,071

=> d his

(FILE 'HOME' ENTERED AT 17:49:08 ON 24 JUL 2003)

FILE 'REGISTRY' ENTERED AT 17:49:18 ON 24 JUL 2003

L1 5 S CTGGATCCTTGCCCCGCCCCTTGAAT|TGGATCCTTGCCCCGCCCCTTGAATT|GGATCCT  
E CYTOSINE/CN  
E CYTOSINE, 5  
E CYTOSINE, 5/CN  
L2 13 S CYTOSINE, 5-METHYL?/CN

FILE 'HCAPLUS' ENTERED AT 18:00:08 ON 24 JUL 2003

L3 128 S REICH N?/AU  
L4 579 S FLYNN J?/AU  
L5 699 S L3 OR L4  
L6 3 S L1  
L7 1537 S L2  
L8 218 S 5(A)METHYL(3A)CYTOSINE  
L9 3 S L5 AND L6  
L10 0 S L6 AND (L7 OR L8)  
L11 3 S L6 AND (METHYLTRANSFERASE# OR METHYL(A)TRANSFERASE#)  
L12 338 S ?MCPG  
L13 0 S L12 AND L6  
L14 266 S METHYL(3A)CPG  
L15 0 S L14 AND L6  
L16 635 S METHYL?(3A)DINUCLEOTIDE#  
L17 1 S L16 AND L6  
L18 62 S L16 AND (METHYLTRANSFERASE# OR METHYL(A)TRANSFERASE#)  
L19 42 S L16 (L) (METHYLTRANSFERASE# OR METHYL(A)TRANSFERASE#)  
L20 1 S L19 AND L6  
L21 3 S L6 OR L9 OR L11 OR L17 OR L20  
L22 3 DUP REM L21 (0 DUPLICATES REMOVED)

=> d que 122

L1 5 SEA FILE=REGISTRY CTGGATCCTTGCCCCGCCCCTTGAAT|TGGATCCTTGCCCCGCCC  
CTTGAATT|GGATCCTTGCCCCGCCCCTTGAATTC|GATCCTTGCCCCGCCCCTTGAATTCC|  
ATCCTTGCCCCGCCCCTTGAATTC|SQSN  
L3 128 SEA FILE=HCAPLUS REICH N?/AU  
L4 579 SEA FILE=HCAPLUS FLYNN J?/AU  
L5 699 SEA FILE=HCAPLUS L3 OR L4  
L6 3 SEA FILE=HCAPLUS L1  
L9 3 SEA FILE=HCAPLUS L5 AND L6  
L11 3 SEA FILE=HCAPLUS L6 AND (METHYLTRANSFERASE# OR METHYL(A)TRANSFE  
RASE#)  
L16 635 SEA FILE=HCAPLUS METHYL?(3A)DINUCLEOTIDE#  
L17 1 SEA FILE=HCAPLUS L16 AND L6  
L19 42 SEA FILE=HCAPLUS L16 (L) (METHYLTRANSFERASE# OR METHYL(A)TRANSF  
ERASE#)  
L20 1 SEA FILE=HCAPLUS L19 AND L6  
L21 3 SEA FILE=HCAPLUS L6 OR L9 OR L11 OR L17 OR L20  
L22 3 DUP REM L21 (0 DUPLICATES REMOVED)

=> d ibib abs 122 1-3

L22 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1999:189269 HCAPLUS

DOCUMENT NUMBER: 130:232472

TITLE: Modulators of DNA cytosine-5 methyltransferase

Lewis 09/485,071

and methods for use thereof  
INVENTOR(S): **Reich, Norbert O.; Flynn, James**  
PATENT ASSIGNEE(S): The Regents of the University of California, USA  
SOURCE: PCT Int. Appl., 114 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

| PATENT NO.  | KIND | DATE     | APPLICATION NO. | DATE        |
|---|------|----------|-----------------|-------------|
| WO 9912027  | A1   | 19990311 | WO 1998-US12351 | 19980612    |
| W: CA, JP, US<br>RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE |      |          |                 |             |
| CA 2300354  | AA   | 19990311 | CA 1998-2300354 | 19980612    |
| EP 1018003  | A1   | 20000712 | EP 1998-930207  | 19980612    |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI                   |      |          |                 |             |
| JP 2001514862   | T2   | 20010918 | JP 2000-508978  | 19980612    |
| US 2003114402   | A1   | 20030619 | US 2001-10476   | 20011207    |
| PRIORITY APPLN. INFO.:  |      |          | US 1997-57411P  | P 19970829  |
|   |      |          | WO 1998-US12351 | W 19980612  |
|   |      |          | US 2000-485071  | A3 20000203 |

AB A synthetic oligonucleotide comprising a C-5 methylcytosine and which recognizes and binds an allosteric site on DNA **methyltransferase** thereby inhibiting DNA **methyltransferase** activity is disclosed. Also disclosed is a compn. comprising a synthetic oligonucleotide of the invention. The compn. is useful for inhibiting DNA **methyltransferase** activity, thereby inhibiting the methylation of DNA. The compn. can be a pharmaceutical compn. useful for treating disorders assocd. with methylation defects, such as cancer and certain developmental disorders. Also disclosed is a method of inhibiting methylation of DNA. The method involves contacting a DNA cytosine-5 **methyltransferase** with a synthetic oligonucleotide of the invention in the presence of the DNA, thereby resulting in an enzyme/synthetic oligonucleotide complex. The presence of the complex prevents catalysis, thereby inhibiting DNA **methyltransferase** activity. Also disclosed is a method of treating a disorder of cell proliferation or development by administering to a subject a synthetic oligonucleotide of the invention. The inhibition of DNA **methyltransferase** prevents the methylation of DNA thereby treating the disorder of cell proliferation or development.

REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1998:387706 HCAPLUS  
DOCUMENT NUMBER: 129:158334  
TITLE: DNA binding discrimination of the murine DNA cytosine-C5 **methyltransferase**  
AUTHOR(S): **Flynn, James; Azzam, Ramzi; Reich, Norbert**  
CORPORATE SOURCE: Department of Chemistry and Program in Biochemistry and Molecular Biology, University of California, Santa Barbara, CA, 93106, USA  
SOURCE: Journal of Molecular Biology (1998), 279(1), 101-116  
CODEN: JMOBAK; ISSN: 0022-2836

PUBLISHER: Academic Press Ltd.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Mammalian DNA cytosine-C5 **methyltransferase** modifies the CpG **dinucleotide** in the context of many different genomic sequences. A rigorous DNA binding assay was developed for the murine enzyme and used to define how sequences flanking the CpG dinucleotide affect the stability of the enzyme:DNA complex. Oligonucleotides contg. a single CpG site form reversible 1:1 complexes with the enzyme that are sequence-specific. A guanine/cytosine-rich 30 base-pair sequence, a mimic of the GC-box cis-element, bound threefold more tightly than an adenine/thymine-rich sequence, a mimic of the cAMP responsive element. However, the binding discrimination between hemi- and unmethylated forms of these DNA substrates was small, as we previously obsd. at the KmDNA level (Biochem., 35, 7308-7315 (1996)). Single-stranded substrates are bound much more weakly than double-stranded DNA forms. An in vitro screening method was used to select for CpG flanking sequence preferences of the DNA **methyltransferase** from a large, divergent population of DNA substrates. After five iterative rounds of increasing selective pressure, guanosine/cytosine-rich sequences were abundant and contributed to binding stabilization for at least 12 base-pairs on either side of a central CpG. Our results suggest a read-out of sequence-dependent conformational features, such as helical flexibility, minor groove dimensions and crit. phosphate orientation and mobility, rather than interactions with specific bases over the course of two complete helical turns. Thus, both studies reveal a preference for guanosine/cytosine deoxynucleotides flanking the cognate CpG. The enzyme specificity for similar sequences in the genome may contribute to the in vivo functions of this vital enzyme.

REFERENCE COUNT: 78 THERE ARE 78 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1996:304202 HCAPLUS  
DOCUMENT NUMBER: 125:4242  
TITLE: Murine DNA Cytosine-C5 **Methyltransferase**:  
Pre-Steady- and Steady-State Kinetic Analysis with  
Regulatory DNA Sequences  
AUTHOR(S): **Flynn, James**; Glickman, J. Fraser;  
**Reich, Norbert O.**  
CORPORATE SOURCE: Department of Chemistry, University of California,  
Santa Barbara, CA, 93106, USA  
SOURCE: Biochemistry (1996), 35(23), 7308-7315  
CODEN: BICHAW; ISSN: 0006-2960  
PUBLISHER: American Chemical Society  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB We present the first description of KmDNA, KdDNA, kcat, and kmethylation for a mammalian DNA **methyltransferase**. Homogeneous, 190 000 Mr DNA (cytosine-5-)-**methyltransferase** isolated from mouse erythroleukemia cells has turnover consts. of 0.15-0.59 h<sup>-1</sup> with single-stranded and unmethylated double-stranded oligonucleotides contg. a single CpG dinucleotide. These substrates were designed to mimic DNA transcriptional cis elements previously reported to have cytosine C-5-methylated regulation. The rate-limiting step for these substrates is the methylation step itself. In contrast, hemimethylated double-stranded substrates show burst kinetics, consistent with a rapid methylation event (3 h<sup>-1</sup>) followed by a slower step which det. steady-state kcat. Hemimethylated and unmethylated double-stranded DNA shows similar binding affinities; these results reveal the mol. basis for the enzyme's

preference for hemimethylated DNA to be the Me transfer step. Substrates with multiple recognition sites do not show burst kinetics and have turnover rate consts. of  $6 \text{ h}^{-1}$ . Catalytic turnover for the mammalian enzyme is thus approx. 20-fold slower than that for the related bacterial enzymes. Our combined results show quant. that one enzyme is certainly capable of both maintenance and de novo methylation and that maintenance of the genomic methylation pattern is preferred over the de novo establishment of new patterns. Direct comparison of the mammalian enzyme with the bacterial DNA cytosine-C5 **methyltransferase**, M.SssI, indicates dramatic differences in preferences for single-stranded, double-stranded, and hemimethylated double-stranded substrates. Moreover, the specificity hierarchy shown for the M.SssI is derived from very different changes in  $K_m$  and catalysis than those obsd. for the mammalian DCMTase. These results demonstrate that the M.SssI, and perhaps other DNA cytosine **methyltransferases** from bacteria, is functionally dissimilar to the mammalian enzyme.